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Application of appropriate immunological method in the identification of the host preference behaviour of vector mosquitoes *Aedes aegypti* Linn and *Culex quinquefasciatus* Say

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Abstract
Mosquitoes tend to alter or change their behaviour quite often in nature. Immunological methods are widely used for the assessment and identification of host selection and host preference behavior of vector and also nuisance mosquitoes. In this context, *Aedes aegypti* Linn and *Culex quinquefasciatus*, the vector mosquitoes were selected to study their host preference pattern at present by using three hosts human, bird and mammals. The dynamic nature and opportunistic behaviour of these mosquitoes were confirmed. Therefore, this study suggests that periodic review of feeding pattern of mosquitoes by using immunological methods is the need of the hour to check the outbreak of vector-borne diseases. Further, cost-effective novel immunological methods shall be involved in the near future too.

Keywords: Immunological technique, host selection, host preference behaviour, mosquitoes

1. Introduction
Mosquitoes are considered as nuisance pests and also act as potential vectors in transmitting diseases like malaria, dengue fever, brain fever and filariasis. Natural cycles of several arboviruses involve different groups of vertebrates with mosquitoes as vectors. The study of the blood feeding behaviour of mosquitoes is essential for understanding the transmission of arboviruses. Knowledge about feeding index of mosquitoes can also be important to define zoo prophylactic approaches to control mosquitoe borne diseases (Tirados *et al.*, 2006). Therefore, surveillance of any mosquitoe-borne disease depends upon not only on the adequate epidemiological information but also upon the entomological aspects of vector especially its feeding and breeding behaviour. The detection of exact vector with reference to a particular host is a paramount importance in the epidemiological aspects of a disease mainly during the epidemic outbreaks and endemic prevalence.

Many zoonotic diseases became human diseases because of the shift in the feeding behaviour of vectors. There are several cues for host selection behaviour which includes the host preference and host selection behavioural pattern. The first major parameter is intrinsic factor and this factor could either be genetic or physiological. The second parameter is availability of hosts which can be divided into accessibility (host density) and vulnerability interactions of host and mosquitoes, which is usually affected by behaviour that are used by the mosquitoes.

Host seeking behaviour is influenced by wide variety of visual, olfactory, gustory and physical stimuli. Any one or combination of these stimuli could potentially act as cue for mosquitoes for host identification and selection. Bernier *et al*., (2000) analyzed 346 human skin emanations, out of which 277 compounds act as attractants for *Aedes aegypti*. Qui *et al*., (2006) found that there is an inter-individual variation towards human odours in feeding pattern of malarial vectors. Sometimes a major determining factor in host selection is the variety of host options or lack of relative host abundance and accessibility often make research difficult when trying to understand exactly how a mosquito chooses its host.

Richards *et al*.,(2000) noted that it was a complicated process to know how local host could affect host preference and reported variations in feeding patterns of two different species of mosquitoes (*Ae. vexans, Oc. triseriatus*) from geographic variation in composition and

abundance /availability of possible mammalian hosts. As an another example, *Aedes albopictus* was an opportunistic feeder, and consequently used to feed on locally abundant and available hosts (Richards, 2006) [1]. This behaviour allowed the species to reproduce successfully and survive effectively regardless of the local host population.

There are roughly 3,200 species of mosquitoes throughout the world (Day *et al*, 2005) and most of them are not anthropophagic and do not pose immediate threat to human. There are few species, which present the formidable issue of disease. *Anopheles* spp, *Aedes spp*, *Culex spp* act as potential vectors in transmitting diseases. In this context, the present study has been undertaken to study the host preference of two species of mosquitoes namely *Aedes aegypti* and *Culex quinquefasciatus* by using immunological tools. By knowing the host preference patterns of the majors, it could be possible to keep barrier between the mosquitoes and man inorder to reduce the transmission of diseases.

2. Materials and Methods:

Larval stages of *Culex quinquefasciatus* and *Aedes aegypti* were collected from their natural breeding habitats. These two species are potential vectors in the urban and rural areas. The larval stages were reared under laboratory conditions in different cages. The newly emerged and unfed adults were used for the experimental purpose. Three types of hosts such as human, guinea pig and hen were selected for the preference study. The preference of the test mosquitoes with respective of the three different hosts was used to find out the feeding index of mosquitoes.

The exposure study was designed in three types, namely single host exposure, double hosts exposure and triple hosts exposure. Blood meals were collected from the fully fed mosquitoes and subjected to blood meal analysis by modified gel diffusion method (Ouchterlony *et al*, 1973) as adapted by (Collins *et al*, 1983). Precoated slides were overlaid with 0.1% agar solution (prepared in Tris glycine buffer) and kept in the refrigerator for three hours before cutting the wells for loading the antisera and blood samples. Fully fed mosquitoes were collected by exposing different hosts such as hen, guinea pig, and human volunteer for about one hour. Blood meal antigen, which was eluted from smears in the Whatman filter paper no. 1 was added in the central well. The antisera of different hosts (hen, guinea pig and human), which were obtained from Serum Institute of India, Calcutta were added in the surrounding wells in the slide. After loading, the slides were kept in a humidity chamber for overnight. The slides were stained with Coomassie blue stain, which produce clear stained bands. The formation of precipitin bands at the interface of antigen and antibody reveals the preference pattern of mosquitoe species. The band look like an arc, which is the resultant product of antigen and antisera. This helps to identify the single or more than one host feeding preference very precisely.

3. Results and Discussion:

Identification of mosquito blood meals is very essential for understanding mosquito feeding behaviour and vector-borne disease transmission possibility. The precipitin ring test was one of the first serological methods employed for identification of mosquito blood meals in the 1920's. Despite of several variations including the agar gel diffusion and microplate tests, the precipitin ring test is highly dependent on the specificity and sensitivity of the antisera developed. As comparing with advanced techniques such as ELISA and cytochrome B mitochondrial gene (Norris, 2005) to identify blood meal sources of mosquitoes, the precipitin technique being simpler and inexpensive hence, the same has been adopted for doing this present work. This method is cost-effective too.

Aedes aegypti showed 77%, 58%, 46% of preference to avian, human and guinea pig respectively in single host exposure (Table.1).It exclusively feeds on hen and avoids guinea pig in double host exposure, whereas it preferred only on human and to lesser extent hen and avoided guinea pig in triple host exposure (Figure.1).The shift from zoophilic to anthrophilic is more obvious within species.

Culex quinquefasciatus exhibited 33 %, 27%, 15% of preference to avian, human and guinea pig respectively (Table.2). When exposed to double hosts, hen and guinea pig simultaneously, it exclusively preferred to feed on hen and avoided guinea pig. In triple hosts exposure, it preferred hen and avoided both human and guinea pig (Figure.1).From the above studies, it is obvious that *Culex quinquefasciatus* seemed to be more of zoophilic in nature.

Studies were conducted elsewhere with regard to host selection pattern of *Aedes aegypti* and *Culex quinquefasciatus*. Generally, *Aedes aegypti* was considered as man –biting species (anthrophilic) but there is evidence from Africa that animals may be the principal host (zoophilic pattern) in some areas. As new selection pressures are applied to *Aedes aegypti* either directly or indirectly, by public health measures, domestic populations of the mosquitoes are capable of changing their feeding habits, resting places and choice of larval habitats as well as their resistance to insecticides. It remains essential to conduct periodic survey on host preference too to assess whether these vectors are potential threat in the epidemic and endemic areas.

(Richards *et al*., 2000) reported that the host preference pattern of *Aedes albopictus* as higher for human (anthrophilic) rather than dogs and cats (zoophilic). It was documented that *Aedes* species used to feed opportunistically and tried to have broad host range. Studies also reported that *Aedes aegypti* and *Aedes albopictus* were highly anthrophilic species in rural village in Tamil Nadu (Tewari *et al*, 2004). In Southern Thailand, *Aedes* species remain as highly anthrophilic both in single and double meals, (Ponlawat and Harrington, 2005). About 92% of the field collected *Aedes albopictus* females fed on blood of mammals indicating high degree of zoophilic pattern (Rui – De Xue *et al*, 2008) [7]. In Southern district of Andaman and Nicobar Islands, Sivan. A *et al*., 2015 [9] reported that both the species *Aedes aegypti* and *Aedes albopictus* readily fed upon human and act as effective vector for diseases such as Dengue and Chikungunya. In this context, the present study reveals that *Aedes aegypti* shows both anthrophilic and zoophilic nature indicating its adaptability to the availability of hosts in nature.

Culex quinquefasciatus feeds on diverse range of hosts involving mammals and birds. It is evident that other mammals like dogs was main source of blood meal for *Culex quinquefasciatus* in the study conducted in Paraiba valley, Brazil (Gomes *et al.*, 2002). Studies also showed that in the Ria De Janeiro of Brazil, *Culex* species fed mostly on mammals (Lovos *et al.*, 2010) [6]. However, *Culex*

quinquefasciatus preferred to feed on human and acted as potential vector of Lymphatic filariasis in Raipur city of Chhattisgarh state (Dixit *et al*., 2000). *Culex quinquefasciatus* act as an opportunistic feeder, feed on birds and mammals (Molaei *et al.*, 2007) [11]. In this context, the present study regarding host preference showed that *Culex quinquefasciatus* preferred avian exclusively indicating its zoophilic nature.

Table 1: Host preference behaviour of *Aedes aegypti* with reference to different hosts

Types of exposure	Hosts	No.of exposed mosquitoes	Fully fed mosquitoes	% of fully fed mosquitoes
Single host exposure	Human Hen Guinea pig	100 59 28	58 46 13	58 77.9 46.4
Double hosts exposure	Hen Guinea pig	16	11	68.7
Triple hosts exposure	Human Hen Guinea pig	57	33	57.8

Table 2: Host preference of *Culex quinquefasciatus* with reference to different hosts

Types of exposure	Hosts	No. of exposed mosquitoes	Fully fed mosquitoes	% of fully fed mosquitoes
Single host exposure	Human Hen Guinea pig	70 67 72	19 22 11	27.1 32.8 15.2
Double hosts exposure	Hen Guinea pig	62	30	32.2
Triple hosts exposure	Human Hen Guinea pig	36	11	30.5

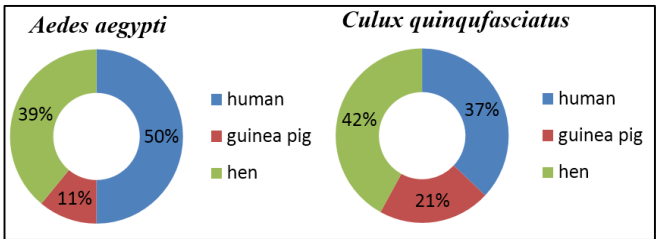

Fig 1: Host preference behaviour of two mosquito species with exposure to single host.

4. Conclusion

The study on the bionomics of vectors is important before the introduction of control methods. It also requires an accurate information about their feeding habits with reference to their host preference. The host preference pattern of vector mosquitoes influences greatly on the dynamics of the transmission of infection in the community. Host selection indicates the host actually fed upon and host preference indicates the innate habit of exercising choice of a host or hosts. Mosquitoes have very intricate patterns dealing with host selection, and research has only uncovered a fraction of what has to be discovered in order to control diseases transmitted by these insects. It is known that environmental factors and genetic predisposition are driving factors for mosquitoes to select one host over the other, yet the intricate details still need to be discovered. To conclude, these insects are extremely important in many aspects – the most important is its capacity to transfer disease. Current and future research has a burden to uncover necessary information, not only to further the knowledge of an insect, but to further knowledge of a disease vector. In this endeavor, immunological method plays a key role. Novel methods need to be involved for better surveillance.

5. References

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